

# Pulmonary Toxicity in Hamsters of Smoke Particles from Kuwaiti Oil Fires

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The Kuwaiti oil wells set on fire by retreating Iraqi troops at the end of the Persian Gulf War released complex particles, inorganic and organic gases, and hydrocarbons into the atmosphere, damaging the environment where many people live and work. In this study, we assessed the health effects of particles from the Kuwaiti oil fires by instilling hamsters intratracheally with particles ( $<3.5 \mu\text{m}$  in size) collected in Ahmadi, a residential area in Kuwait located downwind of hundreds of oil fires. Twenty-four hours after instillation, we performed bronchoalveolar lavage (BAL) to assess various indicators of pulmonary inflammation, including neutrophil and macrophage numbers; albumin, an index of air-blood barrier permeability; and activities of three enzymes: lactate dehydrogenase (LDH; an indicator of cell injury), myeloperoxidase (MPO; which indicates activation of neutrophils), and  $\beta$ -N-acetylglucosaminidase (GLN; which is indicative of damage to macrophages or neutrophils). We compared the response of hamsters instilled with particles from Ahmadi to animals instilled with urban particles collected in St. Louis, Missouri. We also compared the Ahmadi particles against a highly fibrogenic positive control ( $\alpha$ -quartz) and a relatively nontoxic negative control (iron oxide). When compared to hamsters instilled with particles from St. Louis, the animals treated with the Ahmadi particles had between 1.4- and 2.2-fold more neutrophils in their BAL fluids. The Ahmadi hamsters had more macrophages and lower MPO and LDH activities, but comparable albumin levels and GLN activities. Thus, the acute toxicity of the Ahmadi particles was roughly similar to that of urban particles collected in the United States, when identical masses were compared. However, the relatively higher concentrations of particles measured in Kuwait and Saudi Arabia during the oil fires (at times more than 16 times higher than the EPA standard) is of particular concern. In addition, since the long-term effects of exposure to these particles remains unknown, further studies are needed to fully assess the health effects of the Kuwaiti oil fires. **Key words:** air pollution, bronchoalveolar lavage, Gulf War, Kuwait, lung, particles, smoke. *Environ Health Perspect* 106:141–146 (1998). [Online 3 February 1998] <http://ehpnet1.niehs.nih.gov/docs/1998/106p141-146brain/abstract.html>

An environmental disaster occurred at the end of the Persian Gulf War when the Iraqi troops damaged and set fire to over 600 oil wells in Kuwait (1). As the oil burned, complex particles, inorganic and organic gases, and hydrocarbons were released into the environment where many humans lived and worked (1,2). The immense magnitude of the fires and smoke plumes attracted worldwide attention to the Gulf region and raised concerns over their potential health effects (3).

Previous studies have shown that exposure to combustion products can increase the incidence of respiratory symptoms, as well as the risk of death from cardiopulmonary disease (4–14). Daily mortality rises in the days following unusually high levels of smog (4–7). In addition, acute exposure to high levels of particles is associated with an increase in respiratory symptoms in children (8), increased hospital usage, changes in lung function, exacerbation of asthma (9), and preterm delivery (15). Longer term exposures have been associated with increased risk of death from lung cancer and cardiopulmonary disease (10,11), as well as changes in lung function and increased incidence of respiratory symptoms in both children and adults (12–15).

However, little is known about the health effects of exposure to sour crude oil (e.g., Kuwaiti oil), which contains various contaminants including high concentrations of hydrogen sulfide, other sulfur compounds, and many trace metals. All of these constituents have the potential to produce irritating, possibly acidic smoke (16). In addition, open burning may alter the nature of the particles, compared to the controlled combustion that occurs in engines and power plants. Given the deleterious effects of particles, we wanted to determine whether exposure to particles from the Kuwaiti oil well smoke could result in pulmonary inflammation and to compare the response to these particles to particles from other sources.

To assess the relative pulmonary toxicity of the oil well fire particles, we instilled hamsters intratracheally with samples of particulate air pollution collected in the town of Ahmadi, which is a residential area located directly downwind from hundreds of oil fires. It is considered to be the largest population center affected by the oil fires. Samples were collected approximately 9 weeks after the fires began, soon after the cessation of hostilities. To quantify the

inflammatory response to the instillation of these particles, we performed bronchoalveolar lavage (BAL) 24 hr after instillation. We analyzed the BAL fluid to assess six aspects of the inflammatory response: neutrophil and macrophage numbers; albumin, as an index of air-blood barrier permeability; and activities of three enzymes that indicate cell injury (lactate dehydrogenase; LDH), activation of neutrophils (myeloperoxidase; MPO), and damage to macrophages or neutrophils ( $\beta$ -N-acetylglucosaminidase; GLN). We compared the effect of instillation of particles from Ahmadi to that seen in animals instilled with samples of urban particles collected during summer days in St. Louis, Missouri, in 1985. The latter studies were performed on a separate cohort of animals prior to the current study of the Ahmadi particles. To further gauge the toxicity of the Ahmadi particles, we compared the inflammatory response seen after the instillation of these particles to a positive control,  $\alpha$ -quartz, which we and others have previously shown to cause a marked inflammatory response, and a negative control, iron oxide, which we have shown to be relatively nontoxic (17). We also assessed the persistence of the inflammatory response to the Ahmadi particles by comparing levels of inflammatory indicators in the BAL of hamsters 1 day and 7 days after particle instillation.

The same Ahmadi particle samples were also evaluated for genotoxicity because the combustion products of these oil fires were known to contain mutagenic and potentially carcinogenic materials (18,19). These results have been reported separately by Kelsey et al. (20).

## Materials and Methods

**Animals.** The methods used in this study were approved by the Harvard Medical Area Standing Committee on Animals. Male Syrian golden hamsters (body weight 90–125 g) were obtained from the Charles River Breeding Labs (Wilmington, MA) and housed individually with access to food and water *ad libitum*. They were maintained on

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a cycle of 12 hr light: 12 hr dark, at a room temperature of between 22 and 26°C.

**Experimental protocols.** This study consisted of three different experiments. We first compared the inflammatory response of hamsters instilled with three doses of particles from Ahmadi [0.15, 0.75, and 3.75 mg/100 g body weight (bw)] to the same doses of particles from St. Louis. All bioassays were performed within 6 months of when the particles were collected.

Next, we assessed the relative toxicity of the 3.75 mg/100 g bw dose of these air samples in comparison to the same dose of a positive control ( $\alpha$ -quartz), and a negative control (iron oxide). The  $\alpha$ -quartz (Min-U-Sil) was obtained from the Pennsylvania Glass Sand Corporation (Pittsburgh, PA). It was fractionated into respirable particles using an Aerotec Model 2 cyclone separator (BGI, Waltham, MA) and collected onto an 0.8  $\mu$ m polyvinylchloride filter (21). The final dust had a count median diameter (CMD) of 1.3  $\mu$ m and a geometric standard deviation (GSD) of 1.9 (21). The iron oxide was a gift from Pfizer, Inc. (Minerals, Pigments and Metals Division, Easton, PA). The CMD and GSD of the iron oxide were 1.3  $\mu$ m and 1.85, respectively.

Finally, we assessed the persistence of the inflammatory response to Ahmadi particles by measuring inflammatory indicators in the BAL of hamsters 1 day and 7 days after particle instillation.

**Preparation of oil fire and urban air particles from filters.** Bulk particles ( $\leq 3.5$   $\mu$ m in diameter) pooled from 10 days of 24-hr air samples collected from 30 April to 9 May 1991 in a residential area in Ahmadi, Kuwait, were used in this study. Samples were collected using a 15 cfm (425 LPM) Anderson high volume sampler, modified with a BGI pre-separator cyclone (50% cut at 3.5  $\mu$ m; BGI). Teflon-coated 8 in  $\times$  10 in Zefluor filters (Millipore, Bedford, MA) were used to trap the particles. For particles typical of urban air pollution, air samples were collected during summer days in St. Louis in 1985 using the same high volume sampler, cyclone, and filters.

The particles were removed from the filters in the following manner. The filters were peeled from their backings and covered with HPLC-grade methanol. A microprobe connected to a 20-kHz ultrasonic cell disruptor (Heat Systems-Ultrasonics, Inc., Farmingdale, NY) was used to dislodge particles from the filter by submerging it in the methanol just above the filter. When the alcohol became saturated, or black, it was replaced with clean methanol. The extractions from the 10 days of sampling were pooled, concentrated by rotary evaporation, and washed with ethanol three times.

Sonication was repeated as necessary to dislodge attached particles. The final extract was then aliquoted into tared microcentrifuge tubes, which were placed in a lyophilizer to remove the remaining ethanol. Before each experiment, an aliquot of the dried extract was resuspended in 30% ethanol at 250 mg/ml.

#### **Intratracheal instillation of particles.**

The particles were suspended in pyrogen-free saline (PFS; 0.9% NaCl) containing 3% ethanol and 13.3 mg/ml rabbit lung surface active material (SAM) (22). SAM was used to help maintain the particles in suspension and reduce damage due to the instillation of fluid. The instillation of the particles was performed in the following manner. The hamsters were anesthetized lightly with Brevital (Eli Lilly, Indianapolis, IN). Particle suspension (0.15 ml/100 g bw) was instilled intratracheally using a blunted and bent 19-gauge 3-in B-D Yale Luer-Lok needle (Becton Dickinson, Franklin Lakes, NJ) attached to a glass syringe (23). Particle concentrations in the instillate were 0.1, 0.5, and 2.5% (w/v). This regimen resulted in particle exposures of 0.15, 0.75, and 3.75 mg, respectively, per 100 g animal. Controls received PFS and SAM alone.

#### **Lung lavage and sample preparation.**

Animals were anesthetized with Nembutal (pentobarbital; Anpro Pharmaceuticals, Arcadia, CA) and killed by exsanguination by cutting the abdominal aorta. Their lungs were lavaged *in situ* with 12 3-ml washes of filtered physiological saline using external massage (24). The first two washes were collected separately from washes 3 through 12. The lavage fluids were centrifuged at  $400 \times g$  at 4°C for 10 min, and the cell pellets from both pools were combined. The supernatant fraction from the first two washes was sedimented at  $15,000 \times g$  for 30 min and used for enzyme and albumin determinations.

**Cell number determinations.** The numbers and types of cells present in each lavage fluid were determined as follows. A well-mixed sample from each lavage fluid was cytocentrifuged onto microscope slides (Cytospin 2; Shandon Southern Instruments, Sewickley, PA), air dried, and stained with Wright-Giemsa stain (VWB Stat Stain, Brisbane, CA). From these slides, a differential count of 200 cells was performed. The number and size of cells found in a separate aliquot were analyzed by a cell counter (Elzone; Particle Data, Elmhurst, IL). The number of cells greater than 6  $\mu$ m in diameter in each sample, along with the differential count, were used to calculate the total number of neutrophils and macrophages in the lavage fluids. To normalize the distribution of the data, we used

the log of the number of cells recovered for our statistical analyses.

**Biochemical assays.** The albumin content and enzyme activities of the cell-free supernatant from the high-speed spin of combined washes 1 and 2 were measured. All enzyme assays were performed at 25°C. Albumin was determined by measuring binding of the dye bromocresol green at 630 nm with bovine serum albumin as a standard (25). LDH activity was assayed by following the oxidation of NADH in the presence of pyruvate at 340 nm (26). MPO activity was measured spectrophotometrically at 470 nm by monitoring the oxidation of guaiacol to tetraguaiacol in the presence of  $H_2O_2$  (27). One unit equals an increase in absorbance of 1.0/min, which is equivalent to 1 mM substrate converted per minute at 25°C. GLN activity was quantified spectrophotometrically by the method of Woollen et al. (28) at 410 nm with *p*-nitro-phenyl- $\beta$ -*N*-glucosaminide as a substrate at pH 5.0. One unit equals 1 mM substrate cleaved per hour.

**Statistical analysis.** We first analyzed the data from the Ahmadi samples and the St. Louis samples by separate two-way analyses of variance (ANOVA) to assess the effect of instillation of various doses of each type of particles on each group of animals, in comparison to PFS + SAM. We then compared responses of the animals to the two types of particles with an additional ANOVA. Because the hamsters used in the Ahmadi and St. Louis studies were from different cohorts, it was important to compare the saline controls from each group, which we did using an ANOVA. We also used an ANOVA to compare the inflammatory response of hamsters to the 3.75 mg/kg dose of Ahmadi, St. Louis,  $\alpha$  quartz, or iron oxide particles, as well as to assess the time course of the response to the instillation of Ahmadi particles. Data are reported as mean  $\pm$  standard error (SE).

## **Results**

**Ahmadi versus St. Louis.** Figure 1 shows the number of neutrophils in the BAL fluid after the instillation of various doses of particles from Ahmadi or St. Louis. Instillation of particles from either site caused a significant increase in the number of neutrophils in the BAL fluid ( $p = 0.025$  for Ahmadi and  $p \leq 0.0001$  for St. Louis). In comparing the two different types of particles, we found that instillation of Ahmadi particles resulted in significantly higher numbers of neutrophils in the BAL fluid ( $p = 0.002$ ), with the number of these cells recovered ranging from between 1.4 and 2.2-fold higher than the number recovered from hamsters instilled with St. Louis particles. This difference was significant



only at the lowest doses of 0.15 mg/100 g bw ( $p = 0.003$ ). There was no significant difference between the neutrophil levels in the two saline-instilled groups.

The number of macrophages in the lavage fluids is shown in Figure 2. Instillation of particles from Ahmadi or St. Louis caused a significant decrease in the number of macrophages in the lavage fluid ( $p = 0.005$  and  $p \leq 0.0001$ , respectively). Significantly more macrophages were collected in the lavage fluids of hamsters instilled with particles from Ahmadi than those instilled with particles from St. Louis ( $p = 0.015$ ). This difference was significant only at the intermediate dose of 0.75 mg/100 g bw ( $p = 0.0033$ ). There was no significant difference in the number of macrophages recovered in the BAL fluids of the two saline-instilled groups.

The concentrations of albumin in the lavage fluids of hamsters instilled with particles from either Ahmadi or St. Louis are shown in Figure 3. Instillation of either type of particles caused a significant increase in the albumin levels in the BAL fluid ( $p \leq 0.0001$  for particles from either site). However, there was no significant difference between BAL albumin levels of hamsters instilled with either type of particles. There was also no difference in the albumin levels of the two groups of saline-instilled animals.

LDH activity in the lavage fluids of hamsters instilled with particles from Ahmadi or St. Louis are shown in Figure 4. Hamsters instilled with particles from either city underwent a significant increase in BAL LDH activities ( $p = 0.0004$  for Ahmadi and  $p \leq 0.0001$  for St. Louis). However, LDH activities were significantly lower in hamsters instilled with particles from Ahmadi, in comparison to those instilled with particles from St. Louis ( $p < 0.002$ ). This difference was significant only at the two highest doses, with  $p = 0.007$  at the 0.75 mg/100 g bw dose and  $p = 0.0008$  at the 3.75 mg/100 g bw dose. There was no difference in the BAL LDH activities in the two saline-instilled groups.

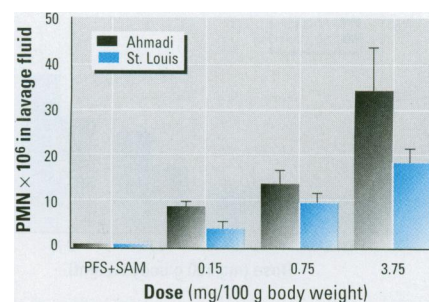
The effect of the instillation of particles from either Ahmadi or St. Louis on MPO activity is shown in Figure 5. BAL MPO activities were significantly higher after the instillation of particles from Ahmadi ( $p = 0.016$ ) and were almost significantly higher in the BAL fluid of hamsters instilled with particles from St. Louis ( $p = 0.058$  vs. controls). In comparing the two groups, the lavage fluid of the hamsters that were instilled with particles from Ahmadi had significantly less MPO activity than did the fluid from hamsters instilled with particles from St. Louis ( $p = 0.005$ ). This difference was significant only at the highest dose of 3.75 mg/100 g bw ( $p = 0.017$ ). However, it

is difficult to assess the significance of this finding because the two saline-instilled groups actually had significantly different levels of MPO activity as well ( $p = 0.001$ ).

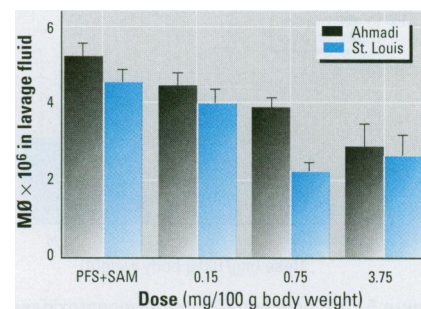
The GLN activities of the BAL of hamsters instilled with particles from either Ahmadi or St. Louis are shown in Figure 6. The instillation of either type of particles caused a significant increase in BAL GLN activity ( $p = 0.02$  for Ahmadi and  $p = 0.0001$  for St. Louis). However, there was no significant difference between the GLN activity of the BAL of hamsters lavaged after instillation of the two types of particles. Again, the interpretation of these results is complicated by the fact that the saline-instilled groups were significantly different from each other ( $p = 0.0001$ ).

**Comparison of air samples with positive ( $\alpha$ -quartz) and negative (iron oxide) controls.** Table 1 shows the neutrophil (PMN) and macrophage (MØ) counts, albumin levels, and LDH, MPO, and GLN activities in hamsters instilled with 3.75 mg/100 g bw of air particles from either Ahmadi or St. Louis, or with  $\alpha$ -quartz (a positive control) or iron oxide (a negative control). In our analysis we compared the contents of the lavage fluids of hamsters instilled with Ahmadi particles to the lavage fluids of the  $\alpha$ -quartz- and iron oxide-instilled animals. The hamsters instilled with Ahmadi particles had significantly more neutrophils and fewer macrophages in their BAL fluids than did the hamsters instilled with iron oxide ( $p = 0.02$  and  $p = 0.005$ , respectively). The numbers of neutrophils and macrophages in the lavage fluid of the Ahmadi hamsters were not significantly different from those seen in the animals that were instilled with  $\alpha$ -quartz. The albumin levels in the BAL of the Ahmadi hamsters were significantly higher than those seen in the BAL of hamsters instilled with either  $\alpha$ -quartz or iron oxide ( $p = 0.0001$  for both treatments). LDH activities of the Ahmadi samples were lower than those seen in the  $\alpha$ -quartz instilled animals ( $p = 0.008$ ) and were not significantly different from the iron oxide-instilled animals. The activities of MPO and GLN in the Ahmadi hamsters fell between the levels seen in hamsters instilled with  $\alpha$ -quartz or with iron oxide, and were not significantly different from either group.

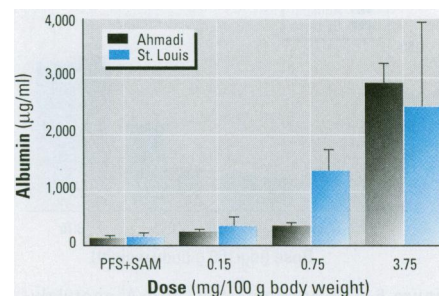
**Time course experiment.** Table 2 summarizes the inflammatory changes seen 1 and 7 days after the instillation of Ahmadi particles. One day after the instillation of particles, all of the inflammatory parameters that we assessed were significantly different from those seen in the control (PFS+SAM) animals. However, by 7 days after instillation, the inflammation was largely resolved.



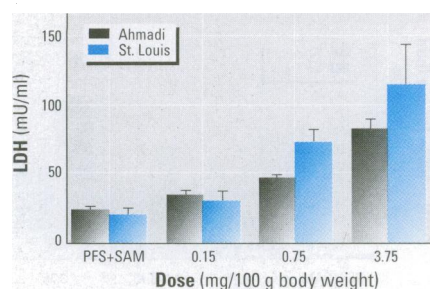
**Figure 1.** The number of neutrophils (PMNs) in the bronchoalveolar lavage fluid collected from hamsters 24 hr after the intratracheal instillation of air particles collected from Ahmadi, Kuwait ( $n = 6$ ), or St. Louis, Missouri ( $n = 6$ ). Because two different cohorts of animals were used in this study, we also compared the control groups [PFS + SAM (pyrogen-free saline + surface active material)]. Control values were not significant; values for Ahmadi versus St. Louis were significant at  $p = 0.002$ .



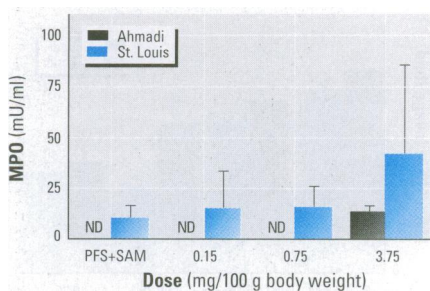
**Figure 2.** The number of macrophages (MØ) in the bronchoalveolar lavage fluid collected from hamsters 24 hr after the intratracheal instillation of air particles collected from Ahmadi, Kuwait ( $n = 6$ ), or St. Louis, Missouri ( $n = 6$ ). Because two different cohorts of animals were used in this study, we also compared the two control groups [PFS + SAM (pyrogen-free saline + surface active material)]. Control values were not significant; values for Ahmadi versus St. Louis were significant at  $p = 0.015$ .



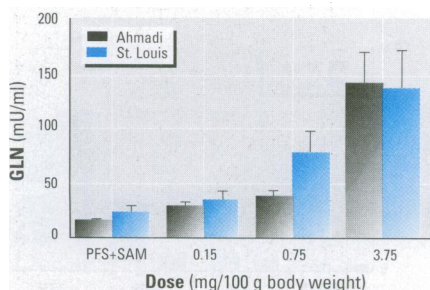
**Figure 3.** The concentration of albumin in the bronchoalveolar lavage fluid collected from hamsters 24 hr after the intratracheal instillation of air particles collected from Ahmadi, Kuwait ( $n = 6$ ), or St. Louis, Missouri ( $n = 6$ ). Because two different cohorts of animals were used in this study, we also compared the two control groups [PFS + SAM (pyrogen-free saline + surface active material)]. Values were not significant for controls or for Ahmadi versus St. Louis.



**Figure 4.** The concentration of lactate dehydrogenase (LDH) in the bronchoalveolar lavage fluid collected from hamsters 24 hr after the intratracheal instillation of air particles collected from Ahmadi, Kuwait ( $n = 6$ ), or St. Louis, Missouri ( $n = 6$ ). Because two different cohorts of animals were used in this study, we also compared the two control groups [PFS + SAM (pyrogen-free saline + surface active material)]. Control values were not significant; values for Ahmadi versus St. Louis were significant at  $p = 0.002$ .



**Figure 5.** The concentration of myeloperoxidase (MPO) in the bronchoalveolar lavage fluid collected from hamsters 24 hr after the intratracheal instillation of air particles collected from Ahmadi, Kuwait ( $n = 6$ ), or St. Louis, Missouri ( $n = 6$ ). Because two different cohorts of animals were used in this study, we also compared the two control groups [PFS + SAM (pyrogen-free saline + surface active material)]. ND, not detectable. Control values were significant at  $p = 0.001$ ; values for Ahmadi versus St. Louis were significant at  $p = 0.005$ .



**Figure 6.** The concentration of  $\beta$ -N-acetylglucosaminidase (GLN) in the bronchoalveolar lavage fluid collected from hamsters 24 hr after the intratracheal instillation of air particles collected from Ahmadi, Kuwait ( $n = 6$ ), or St. Louis, Missouri ( $n = 6$ ). Because two different cohorts of animals were used in this study, we also compared the two control groups [PFS + SAM (pyrogen-free saline + surface active material)]. Control values were significant at  $p = 0.001$ ; values for Ahmadi versus St. Louis were not significant.

**Table 1.** Ahmadi particles versus  $\alpha$ -quartz and iron oxide

	PMN ( $\times 10^6$ cells)	MØ ( $\times 10^6$ cells)	Albumin ( $\mu$ g/ml)	LDH (mU/ml)	MPO (mU/ml)	GLN (mU/ml)
Ahmadi, Kuwait	$34.32 \pm 9.43$	$2.93 \pm 0.44$	$2,825.40 \pm 361.07$	$80.80 \pm 6.48$	$12.68 \pm 3.06$	$143.28 \pm 29.82$
St. Louis, MO	$18.56 \pm 2.71$	$2.67 \pm 0.56$	$2,418.83 \pm 614.50$	$113.05 \pm 12.00$	$41.90 \pm 17.50$	$139.51 \pm 14.00$
$\alpha$ -Quartz	$37.29 \pm 9.11$	$3.68 \pm 0.46$	$654.01 \pm 186.32$	$112.74 \pm 6.43$	$32.98 \pm 5.48$	$187.5 \pm 28.41$
Iron oxide	$14.67 \pm 3.32$	$5.87 \pm 0.57$	$239.00 \pm 30.82$	$68.44 \pm 6.26$	$5.31 \pm 2.11$	$86.51 \pm 10.20$
Ahmadi vs. $\alpha$ -quartz	NS	NS	$p = 0.0001$	$p = 0.008$	NS	NS
Ahmadi vs. iron oxide	$p = 0.02$	$p = 0.005$	$p = 0.0001$	NS	NS	NS

Abbreviations: PMN, neutrophils; MØ, macrophage; LDH, lactate dehydrogenase; MPO, myeloperoxidase; GLN,  $\beta$ -N-acetylglucosaminidase; NS, not significant.

**Table 2.** Effect of Ahmadi particles 1 day and 7 days after instillation

	PMN ( $\times 10^6$ cells)	MØ ( $\times 10^6$ cells)	Albumin ( $\mu$ g/ml)	LDH (mU/ml)	MPO (mU/ml)	GLN (mU/ml)
Control	$0.65 \pm 0.19$	$5.35 \pm 0.36$	$128.58 \pm 7.11$	$22.63 \pm 3.14$	$0.00 \pm 0.00$	$15.20 \pm 1.22$
Day 1	$34.32 \pm 9.43$	$2.93 \pm 0.44$	$2,825.40 \pm 361.07$	$80.80 \pm 6.48$	$12.68 \pm 3.06$	$143.28 \pm 29.82$
Day 7	$2.32 \pm 0.80$	$9.99 \pm 0.53$	$170.84 \pm 15.83$	$37.97 \pm 4.63$	$0.17 \pm 0.14$	$22.39 \pm 1.81$
Control vs. day 1	$p = 0.0001$	$p = 0.0003$	$p = 0.0001$	$p = 0.0001$	$p = 0.0001$	$p = 0.0001$
Control vs. day 7	$p = 0.02$	$p = 0.0004$	NS	$p = 0.04$	NS	NS

Abbreviations: PMN, neutrophils; MØ, macrophage; LDH, lactate dehydrogenase; MPO, myeloperoxidase; GLN,  $\beta$ -N-acetylglucosaminidase; NS, not significant.

The number of neutrophils in the BAL had fallen, but remained higher than in the control animals ( $p = 0.02$ ). Likewise, LDH activity remained just slightly elevated beyond the controls ( $p = 0.04$ ). Albumin levels and MPO and GLN activities had returned to levels seen in the control animals. The one parameter that was strikingly different from both the control and day 1 animals was the number of macrophages. Alveolar macrophage numbers, which fell one day after particle instillation, had increased almost twofold beyond normal levels by day 7 ( $p = 0.0004$ ).

## Discussion

This paper presents results of a short-term animal bioassay designed to estimate the pulmonary toxicity of smoke particles from the oil well fires in Kuwait. We found that smoke particles from Kuwait are toxic, resulting in an inflammatory response that is comparable to that seen in animals instilled with particles from St. Louis or with  $\alpha$ -quartz, our positive control. The concentrations of particles released into the atmosphere during the Kuwaiti oil fires greatly exceeded the levels experienced by residents of an American city, potentially increasing the likelihood that people exposed to smoke from these fires may have suffered more serious health consequences. On the other hand, urban residents are exposed to low levels of these relatively toxic particles daily over the course of their lifetimes, while the Kuwaiti oil fires represented a single incident, which was largely resolved within a period of months.

One day after particle instillation, there was an increase in the number of neutrophils

in the BAL. We also observed elevated MPO activity in the BAL fluid at the highest dose of particles, indicating that the neutrophils were activated and releasing this enzyme. In addition, the number of alveolar macrophages in the BAL fluid decreased. This may be the result of the cytotoxicity of these particles, a phenomenon that we have previously observed with  $\alpha$ -quartz (17). There was also evidence of increased permeability of the air–blood barrier because BAL levels of albumin were elevated. Finally, the increased LDH and GLN activities indicate that cellular damage had occurred.

In comparing the Ahmadi hamsters to hamsters instilled with particles collected in St. Louis, which we considered to be characteristic of summer daytime urban air pollution in the United States, we found that animals treated with the Ahmadi particles had between 1.4 and 2.2-fold more neutrophils in their BAL fluids than did animals that were instilled with St. Louis particles. Given the larger number of neutrophils in the BAL of the Ahmadi hamsters, we were surprised to find significantly higher levels of MPO in the lavage fluid of hamsters instilled with particles from St. Louis than from Ahmadi. However, because the saline-instilled animals from the St. Louis group had significantly higher levels of MPO than did the Ahmadi group, it is difficult to know how to interpret these findings.

Both groups of hamsters showed comparable degrees of increased air–blood barrier permeability, as indicated by their similar BAL albumin levels. Likewise, the fact that both groups had comparable GLN activities suggests that animals treated with particles from either site had similar degrees of damage



to neutrophils and macrophages. However, the higher levels of LDH activity in the BAL of the St. Louis hamsters suggests that these particles caused more overall cell damage.

Previous experiments with highly fibrogenic  $\alpha$ -quartz and relatively nontoxic iron oxide demonstrated that this bioassay could differentiate among particles with different toxicities as based on human epidemiological and chronic animal studies (17). By comparing the test sample with these positive and negative controls, the pulmonary toxicity of uncharacterized samples may be estimated. For example, this system was applied to Mt. St. Helens ash (29) and to di(2-ethylhexyl) sebacate (30), both of which were found to have relatively low pulmonary toxicity. In contrast, particles collected on summer days in St. Louis were found to produce dramatic increases in toxic effects. The goal of this portion of the present study was to determine the relative toxicity of the particles collected in Ahmadi's environment.

Instillation of particles from Ahmadi caused a similar degree of pulmonary inflammation, as assessed by BAL parameters, as  $\alpha$ -quartz particles. Both particle types caused similar degrees of neutrophil influx into the BAL and a comparable decrease in the number of macrophages recovered in the BAL. The response to the two types of particles differed in that instillation of Ahmadi particles caused a significantly greater increase in albumin levels, but a smaller change in BAL LDH activity, than instillation of  $\alpha$ -quartz. The Ahmadi particles were more toxic than our inert control, iron oxide dust, causing more neutrophils, fewer macrophages, and higher albumin levels in the BAL.

By assessing changes in the magnitude of the inflammatory response over time, we found that the inflammation induced by the instillation of Ahmadi particles was beginning to return to levels seen in control (PFS+SAM) animals by 7 days after instillation. We noted that the number of macrophages in the lavage fluid, which was decreased 1 day after instillation of Ahmadi particles, was significantly elevated after 7 days to levels almost twofold higher than seen in the controls. Thus, macrophage toxicity is supplanted by macrophage recruitment, a sign of chronic inflammation (17).

While the acute toxicity of Ahmadi particles was relatively similar to that of urban air pollution in the United States, particle concentrations at the Ahmadi residential site were greater than the values generally observed in the United States. The EPA has established  $150 \mu\text{g}/\text{m}^3$  as the  $\text{PM}_{10}$  (particles  $<10 \mu\text{m}$  in aerodynamic diameter) standard for a 24-hr period.  $\text{PM}_{10}$  concentrations at the Ahmadi site averaged  $343 \pm 65 \mu\text{g}/\text{m}^3$

over the course of the sampling period and were consistently above  $190 \mu\text{g}/\text{m}^3$ .  $\text{PM}_{10}$  levels in Northern Saudi Arabia, near the border with Kuwait, reached more than  $2,500 \mu\text{g}/\text{m}^3$  in May of 1991 (31). Wind-blown dust elevates the concentration of air pollution particles, even in the absence of oil well fires. These particles tend to be larger than  $3.5 \mu\text{m}$  aerodynamic diameter and are generally deposited in the upper respiratory tract; thus, they are cleared relatively easily. Oil well fire particles are in the smaller size range, usually less than  $3.5 \mu\text{m}$  mass median aerodynamic diameter, which is the size range that was sampled for these pulmonary toxicity evaluations.

The health consequences of exposure to smoke from the Kuwaiti oil fires have been studied in a limited number of human and animal studies. The effect of smoke exposure on pulmonary function of military personnel was assessed in a prospective cohort study of 125 men (mean age 26 years) who were part of a squadron of British bomb disposal engineers working in Kuwait (31). Pulmonary function, including forced expiratory volume in one second ( $\text{FEV}_1$ ), forced expiratory flow 25–75% ( $\text{FEF}_{25-75\%}$ ; the middle 50% of  $\text{FEV}_1$ ), and forced vital capacity (FVC) were measured in the men before their deployment and once every 2 weeks during their 5-month stay, from June to October 1991. Coombe (31) focused on the  $\text{FEF}_{25-75\%}$  as the most sensitive measure of early small airway changes, but found no significant difference in this value over the course of the exposure period.

Blood levels of organic compounds from the smoke from the oil fires and the genotoxic consequences of exposure to these agents have been assessed by several groups. Etzel and Ashley (32) reported higher blood levels of organic compounds in the blood of firefighters who fought the oil fires than in a reference group in the United States. However, levels of these compounds in a separate cohort of U.S. military personnel who were working in Kuwait City but were not involved in fighting the fires were no higher than those seen in the reference group.

The same Ahmadi particle samples that were used in the current study were also used to assess *in vitro* genotoxicity (20). The particles were used in three standard genotoxic assays: sister chromatid exchanges (SCE) in human peripheral blood lymphocytes, mutation at the *hprt* locus in the human lymphoblast cell line AHH-1, and DNA adduct formation in AHH-1 cells. The results were compared to those seen in response to a reference sample of particles from urban air collected in Washington,

D.C. Kelsey et al. (20) found an increase in SCE and in mutations in cells exposed to particles from Kuwait in comparison to the reference sample. However, they found no increase in the formation of DNA adducts in cells exposed to the Kuwaiti particles.

Similar results were seen in humans exposed to oil smoke while working in Kuwait during the fires. McDiarmid et al. (19) studied the frequency of SCE in peripheral blood lymphocytes collected before, during, and after their deployment to Kuwait. They found an increase in SCE in samples collected 2 months into the troops' deployment in Kuwait. The rate of SCE remained high in samples collected 1 month after the soldiers returned to Germany. Darcey et al. (18) studied levels of aromatic DNA adducts in nine men before and after they went to Kuwait to fight the oil fires. Despite the fact that the men had been uniformly exposed to the plumes of smoke both during fire fighting and at their residence adjacent to the burning oil fields, only one man had elevated levels of DNA adducts. Darcey et al. (18) concluded that exposure of the firefighters to aromatic compounds was of limited extent during the oil fires.

Taken together, these data suggest that the acute toxicity of the particles found in the smoke from the Kuwaiti oil fires is comparable to that of urban particles. Thus, they may contribute to both acute and chronic cardiopulmonary disease and death. These particles are also genotoxic; thus, exposure to smoke from the fires may increase SCE. The consequences of these genetic changes may manifest themselves in the future, in the form of an increased incidence of cancer. Most epidemiological studies of the effects of these particles have been performed on healthy members of the armed services or fire fighting crews and may not be representative of the citizens of Kuwait who were exposed to this smoke, including children, the elderly, and those with preexisting cardiopulmonary disease. Finally, these particles remain in the Kuwaiti environment, and some continue to be resuspended by wind, vehicular traffic, and other forces. Therefore, further studies of the long-term effects of exposure to these particles are needed to fully assess the health effects of exposure to Kuwaiti oil fire smoke.

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## Big Ideas for your Health and Future



- An "environmental genome" study of how disease-susceptibility genes vary from person to person in a representative sector of the population. To help us see why you might get a nerve disorder from an exposure to a chemical whereas your friend Lee might not . . . Information to protect you *and* Lee, without guesstimates and fudge factors.
- A survey of chemicals polluting our blood — a look at what we, as a population, have been exposed to.
- Customized mice to quickly screen chemicals and drugs . . . and help conquer diseases like breast cancer.
- A study of mixtures. We don't face chemicals one on one. Why should science?

These ideas are Now at the National Institute of Environmental Health Sciences, one of the National Institutes of Health, and at the National Toxicology Program, which is headquartered at NIEHS.

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